

PATENT
USSN 09/872,183
Docket 094/004d

CLAIM AMENDMENTS

1 to 20. **CANCELLED**

21. *(Currently amended)* A method for producing a neural cell population from human embryonic stem (hES) cells, comprising culturing progeny of the ~~ES~~ hES cells in a medium containing one or more added neurotrophins and one or more added mitogens, thereby producing a population in which at least 60% of the cells express A2B5, polysialylated NCAM, MAP-2, or Nestin.
22. *(Previously presented)* The method of claim 21, wherein the added neurotrophins include neurotrophin 3 (NT-3) or brain-derived neurotrophic factor (BDNF).
23. *(Previously presented)* The method of claim 22, wherein the added neurotrophins include both NT-3 and BDNF.
24. *(Previously presented)* The method of claim 21, wherein the added mitogens include a mitogen selected from epidermal growth factor (EGF), basic fibroblast growth factor (bFGF), platelet-derived growth factor (PDGF), and insulin-like growth factor 1 (IGF-1).
25. *(Previously presented)* The method of claim 24, wherein the added mitogens include both PDGF and IGF-1.
26. *(Previously presented)* The method of claim 21, wherein the progeny are cultured on fibronectin.

27 to 29. **CANCELLED**

30. *(Previously presented)* The method of claim 21, comprising selecting and propagating cells that are positive for polysialylated NCAM.
31. *(Previously presented)* The method of claim 21, wherein the produced cell population is at least 60% positive for polysialylated NCAM.
32. *(Previously presented)* The method of claim 21, wherein the produced cell population is at least 90% positive for polysialylated NCAM.
33. *(Previously presented)* The method of claim 21, wherein the produced cell population is at least 38% β -tubulin III positive.

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34. *(Previously presented)* The method of claim 21, wherein the produced cell population has the characteristic that culturing it in the presence of neurotrophins maintains capacity of the cells to proliferate and to form tyrosine hydroxylase positive neurons upon differentiation.
35. *(Previously presented)* The method of claim 21, wherein the produced cell population is essentially free of undifferentiated hES cells.
36. *(Previously presented)* The method of claim 21, further comprising differentiating the cells into a population that contains at least 30% neurons.
37. *(Previously presented)* The method of claim 21, further comprising differentiating the cells into a population that contains at least 30% MAP-2 positive cells.
38. *(Previously presented)* The method of claim 37, wherein at least 3% of the differentiated cells positive for MAP-2 are also positive for tyrosine hydroxylase.
39. *(Previously presented)* The method of claim 37, wherein at least 8% of the differentiated cells positive for MAP-2 are also positive for tyrosine hydroxylase.
40. *(Previously presented)* The method of claim 37, wherein the differentiating comprises culturing the cells in a medium containing one or more factors selected from neurotrophins, cAMP, and ascorbic acid for at least 3 days, in the absence of added mitogens.
41. *(Previously presented)* The method of claim 21, wherein the differentiated cell population comprises sensory or motor neurons.
42. *(Previously presented)* The method of claim 21, wherein the differentiated cell population comprises oligodendrocytes or astrocytes.
43. *(Withdrawn)* The method of claim 21, further comprising combining the cell population with a compound, determining any phenotypic or metabolic changes in the cell that result from contact with the compound, and correlating the change with cellular toxicity or modulation caused by the compound.
44. *(Withdrawn)* The method of claim 43, comprising determining whether the compound is toxic to cells in the population, or affects ability of cells in the population to be maintained in culture.

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45. *(Withdrawn)* The method of claim 43, comprising determining whether the compound changes neurotransmitter synthesis, release, neurotransmitter uptake, or electrophysiology by cells in the population.
46. *(Previously presented)* The method of claim 21, further comprising combining the cells with a pharmaceutically compatible excipient.

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47. (New) A method for producing a neural cell population from human embryonic stem (hES) cells, comprising initiating differentiation of the hES cells into a heterogeneous population of precursor cells, then culturing the initiated cells in a medium containing one or more added neurotrophins and one or more added mitogens, thereby producing a population in which at least 60% of the cells express A2B5, polysialylated NCAM, MAP-2, or Nestin.
48. (New) The method of claim 47, wherein the added neurotrophins include neurotrophin 3 (NT-3) or brain-derived neurotrophic factor (BDNF).
49. (New) The method of claim 48, wherein the added neurotrophins include both NT-3 and BDNF.
50. (New) The method of claim 47, wherein the added mitogens include a mitogen selected from epidermal growth factor (EGF), basic fibroblast growth factor (bFGF), platelet-derived growth factor (PDGF), and insulin-like growth factor 1 (IGF-1).
51. (New) The method of claim 50, wherein the added mitogens include both PDGF and IGF-1.
52. (New) The method of claim 47, wherein the progeny are cultured on fibronectin.
53. (New) The method of claim 47, wherein differentiation is initiated by culturing the hES cells in a medium containing retinoic acid before culturing with the neurotrophins and mitogens.
54. (New) The method of claim 47, wherein differentiation is initiated by culturing the cells as embryoid bodies before culturing with the neurotrophins and mitogens.
55. (New) The method of claim 47, wherein differentiation is initiated by culturing the cells as cell aggregates before culturing with the neurotrophins and mitogens.
56. (New) The method of claim 47, comprising selecting and propagating cells that are positive for polysialylated NCAM.
57. (New) The method of claim 47, wherein the produced cell population is at least 60% positive for polysialylated NCAM.
58. (New) The method of claim 47, wherein the produced cell population is at least 90% positive for polysialylated NCAM.

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59. (New) The method of claim 47, wherein the produced cell population is at least 38% β -tubulin III positive.
60. (New) The method of claim 47, wherein the produced cell population has the characteristic that culturing it in the presence of neurotrophins maintains capacity of the cells to proliferate and to form tyrosine hydroxylase positive neurons upon differentiation.
61. (New) The method of claim 47, wherein the produced cell population is essentially free of undifferentiated hES cells.
62. (New) The method of claim 47, further comprising differentiating the cells into a population that contains at least 30% neurons.
63. (New) The method of claim 47, further comprising differentiating the cells into a population that contains at least 30% MAP-2 positive cells.
64. (New) The method of claim 63, wherein at least 3% of the differentiated cells positive for MAP-2 are also positive for tyrosine hydroxylase.
65. (New) The method of claim 63, wherein at least 8% of the differentiated cells positive for MAP-2 are also positive for tyrosine hydroxylase.
66. (New) The method of claim 63, wherein the differentiating comprises culturing the cells in a medium containing one or more factors selected from neurotrophins, cAMP, and ascorbic acid for at least 3 days, in the absence of added mitogens.
67. (New) The method of claim 47, wherein the differentiated cell population comprises sensory or motor neurons.
68. (New) The method of claim 47, wherein the differentiated cell population comprises oligodendrocytes or astrocytes.
69. (New) The method of claim 47, further comprising combining the cells with a pharmaceutically compatible excipient.